Temporal monitoring of an SPIO labeled, optically traceable tumor mouse model

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Introduction:

In this work, we present a dual-labeled mouse tumor model that allows monitoring of cells over time. A cell line of human melanoma cancer cells that constitutively expresses enhanced green fluorescent protein (EGFP) was incubated with superparamagnetic iron oxide (SPIO) particles. The cells were optically traceable using multispectral optical imaging [1], providing a true measure of viability as only live and functioning cells produce the fluorescent protein. Furthermore, the cells were detectable by MR imaging techniques sensitive to susceptibility-based contrast. The tissue was observed using the two complementary imaging modalities over a period of approximately 4 weeks and histology was correlated with imaging results at different time points to examine the possible paths of the SPIO particles over time.

Methods:

Human melanoma M21 carcinoma cells were incubated with Feridex (Berlex Laboratories), a superparamagnetic iron oxide (SPIO) in protamine sulfate[2]. Tumor cells were also transfected to constitutively express the EGFP gene, making cells from the tumor line optically fluorescent. Labeled tumors cells were subcutaneously inoculated into the right and/or left flank of five Balb/c nu/nu nude mice and imaged with MR and multispectral optical imaging 7,11,14,and18 days post injection. All MRI was performed on a 3.0T Intera (Philips Medical Systems, Best, The Netherlands) using a dedicated solenoid mouse coil (Philips Research Laboratories, Hamburg, Germany). One mouse was imaged 25 days post injection. Another set of mice was imaged only prior to the time of sacrifice and tissue from all mice was collected for histology. Mice were anesthetized with isoflurane and temperature and breathing were monitored during MR imaging. Histological staining using a Prussian blue stain was performed to highlight the presence of iron within the tumor tissue. The tumors were characterized using multiple MRI techniques including: 1) 2D T_2-weighted (T_2W) turbo-spin echo (TSE) with a spatial resolution of 100x100x500µm (200x200µm actual), 10 echoes, TR=4.5s, TE =70ms,ΔTE=1ms, 6-8 averages; 2) Multi-gradient echo based T_2* mapping: 13 echoes, spatial resolution of 310x310x500µm (420x380µm actual), TR=3.5s,TE_1=5.5ms, ΔTE=2.0ms, 8 avgs. 3) Multi-spin echo based T_2 mapping: 10 echoes, 350x350x500µm, TR=1.8s, TE_1=10ms, ΔTE=10ms, 3 avgs. Whole-body multispectral optical imaging was performed to validate the origin and viability of tumor cells using a Maestro® System (CRI, Inc., Woburn, MA, USA). Exposures lasting 2 s using a 480 nm excitation wavelength were acquired at 5 nm increments from 500 to 600 nm. The system's software was used to remove background autofluorescence, highlighting only the tumor tissue.

Results:

Figure 1 displays the T_2W images of the mice at 7,11,14,18 and 25 days post inoculation with the iron oxide labeled tumor cells. Initially, only fluid is visible in the MR images (not shown). As time progresses, the tumor MR signal demonstrates signal voids probably due to the presence of iron oxide in the tissue. The general shape and form of these features is maintained throughout the multiple time points (Fig 1). Also, an area of signal void very close to the original inoculation site yet outside the tumor remained visible in all imaging time points (images not shown). T_2* and T_2 parametric maps through a tumor slice are shown in Fig 2. The decreased T_2* and T_2 observed in the areas of the tumor indicate the presence of SPIOs in the tumor tissue. Histologic examination of the tissue demonstrates large amounts of iron deposited outside the tumor periphery with much smaller fractions within cells (Fig 3a&b). Multispectral optical imaging (Fig 3c&d) confirms the tumor cells lines beyond the 11th day post injection. On earlier days, the presence of the SPIOs in the tissue greatly reduced the optical signal.

Discussion:

Tracking of cells incubated with SPIOs has been proposed as a non-toxic method to achieve *in vivo* cell tracking due to the high sensitivity of MR to the susceptibility artifacts created by the presence of iron in the tissue [7]. In this work, we demonstrate a dual-labeled tumor model that is both optically and MR detectable, and may be extendable to stem cell research. The cells were imaged over a period of several weeks, and the iron signal was detectable throughout.

Initial histological analysis demonstrated significant amounts of iron in the periphery of the tumor, which may imply loss of iron over time due to cell death or other cellular processes. Furthermore, uptake of iron by cells of mouse origin can take place, putting in question the source of the iron detected with MR. The dual-labeled model coupled with histology can help resolve these questions. Further histological analysis is necessary to identify the source of the iron signal within the tumor.

Our previous work has shown that a large fraction of cells die upon inoculation, as confirmed by optical imaging [6]. In this model, multispectral optical imaging acts as a true measure of cell viability since only living cells producing EGFP will be detected after autofluorescence substaction [6]. The fraction of cells that continues to grow and divide may lose its iron as time progresses either by passive methods or by simple dilution through the process of cell division, making optical tracking a viable option.

This dual-labeled model presents an excellent platform for the molecular study of tumor processes as well as a step in the development of true *in vivo* cell tracking.

References:

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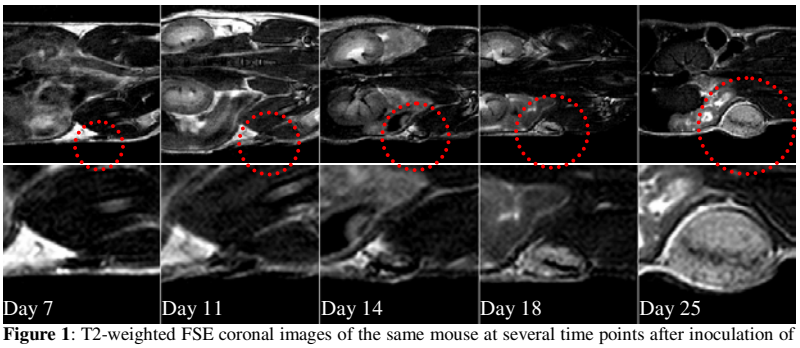

Figure 1: T2-weighted FSE coronal images of the same mouse at several time points after inoculation of iron oxide-labeled M21 tumor cells. Bottom row is a magnified version of the top row. Initially little is visible in these T2-weighted images other than fluid accumulation and a general area of signal loss due to the concentrated iron oxide particles and cells. Throughout tumor development, general features are still identifiable (dark band across tumor) and demonstrate the presence of iron oxide. However, as the tumor continues to grow and enters stages in which necrosis become common (day 25), the signal intensity becomes heterogeneous and it is not possible to attribute signal voids to SPIOs without further histological analysis.

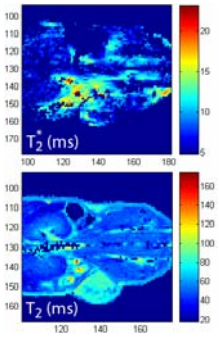

Figure 2: Parametric T_2* (top) and T_2 (bottom) maps of the same mouse in Fig 1 obtained on day 25 post injection. Note consistent features with those obtained from T2W imaging.

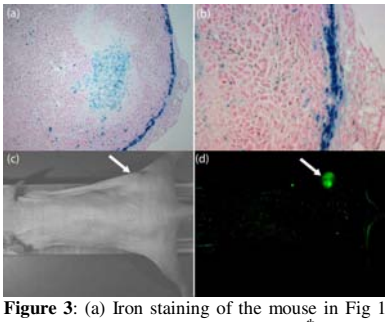

Figure 3: (a) Iron staining of the mouse in Fig 1 performed on tissue preserved on the 25th day after inoculation. Note high concentration of iron in the tumor periphery as well as in the necrotic core. (b) Magnified version of peripheral tumor tissue in (a). (c) White light image and (d) isolated EGFP image from multispectral optical imaging. Tumor tissue is clearly highlighted (arrows).